IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of :
SEKIGUCHI ET AL :
Serial No. 10/812,075:
Examiner: Cecilia M. Jaisle : Art Unit 1624
For: NOVEL QUINOLINE, TETRAHYDROQUINAZOLINE, AND PYRIMIDINE DERIVATIVES AND METHODS OF TREATMENT RELATED TO THE USE THEREOF

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DECLARATION

Assistant Commissioner of Patents, Washington D.C.
Sir:
I, Kanuma Kosuke hereby declare the following:

1. I am a citizen of Japan residing at 1436-1-D101 Nishiowa, Washimiya-machi, Kitakatsushika-gun, Saitama 340-0206, Japan. I graduated from Tokyo University of Science, Tokyo, Japan in March 1994. Since April 1994, I have engaged in Medical Research Laboratories, Taisho Pharmaceutical Co., Ltd.

2. I am a member of the co-inventors of the instant application, and know well the substance of the said patent application.

3. In order to show that the present invention is unpredicted from Wustrow et al. (J. Med. Chem. 41(5):760-771; "Wustrow") which does not disclose that compounds have any significant antagonist activity for the human MCH1 receptor, I conducted the following experiments to compare simultaneously the compounds 36 and 37 in Table 2 at page 764 of "Wustrow" with the compounds of Examples 18 and 19 disclosed in the present invention with respect to the antagonist activity for the human MCH1 receptor.

"Wustrow" cited by the Examiner does not disclose "RN 204245-70-5, 2-Pyrimidinamine, N-[4-[2-methyl(phenylmethyl)amino]ethyl]cyclohexyl]-, trans-". I believe that the structurally closest compounds to this compound in "Wustrow" is "N-(trans-4-(2-(methyl(phenethyl)amino)ethyl)cyclohexyl)pyrimidin-2-amine" which is compound 36 in Table 2 at page 764, so I synthesized compound 36 to evaluate the antagonist activity for the human MCH1 receptor.

Furthermore, "RN 204245-89-6, 2-Pyrimidinamine, N-[4-[2-(dipropylamino)ethyl]cyclohexyl]-, trans-" cited by Examiner is identical with compound 37 in Table 2 at page 764 of "Wustrow", so I synthesized compound 37 to evaluate the antagonist activity for the human MCH1 receptor.

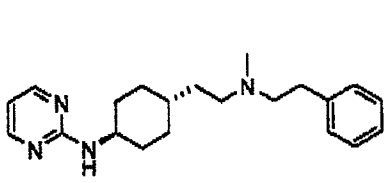
I believe Examples 18 and 19 disclosed in the present invention are the closest compounds to the compounds 36 and 37 of "Wustrow".

4. Experimental Example 1

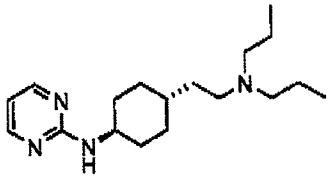
Method

HEK293 cells stably expressing constitutively activated human MCH1 receptor were seeded $3x10^4$ cells/100 μL of complete culture media (Dulbecco's modified Eagle medium with 10 % fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.5 mg/mL G418) into poly-D-lysine pretreated black/clear bottom plates 24 hrs before the assay. Prior to the assay, the media were removed and HEK293 cells were loaded with 2 μM Fluo-4/AM calcium-sensitive dye in loading buffer (Hank's balanced salt solution supplemented with 0.5 mM probenecid, 0.05 mg/mL Amaranth, 20 mM HEPES, pH 7.4, and 0.2 % bovine serum albumin) for 1 hr at 37 °C in a 5 % CO_2 incubator. The loading buffer was removed, and fresh buffer containing various concentrations of the compound (Example 18, 19, Compound 36 or 37) was added to the cells, and the cells were incubated for 30 min at 37 °C. Fluorescence emission caused by increases in intracellular calcium mobilization elicited by 50 nM MCH was measured with FDSS6000™ system.

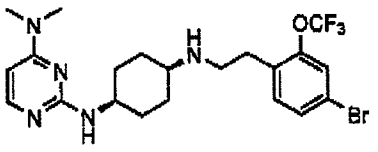
The chemical structures of the evaluated compounds are shown below.



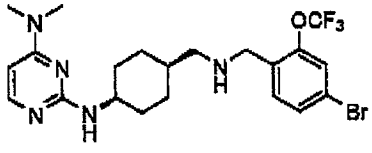
Compound 36



Compound 37



Example 18



Example 19

Result

Compounds 36 and 37 of "Wustrow" did not show any significant antagonist activity for the human MCH1 receptor at a concentration of 10 μM (IC_{50} >10 μM). In contrast, the compounds in the present invention (Examples 18 and 19) demonstrated remarkable and unexpectedly superior antagonist activity for the human MCH1 receptor.

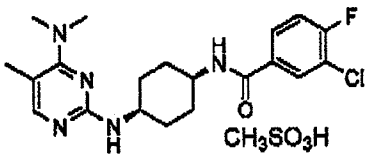
Compound	IC_{50} (nM)
Compound 36	**> 10000**
Compound 37	**> 10000**
Example 18	**49.4**
Example 19	**109**

5. The third compound cited by the Examiner "RN189153-07-9, Cyclohexaneacetic acid, 4-(2-pyrimidinylamino)-, ethylester, trans, is identical with Compound 31 in Scheme 6 on page 762 in "Wustrow". Clearly seen in this scheme, Compound 31 is an intermediate of the final product, compounds 34-48. Among Compounds 34-48, the structurally closest compounds to the present invention are compounds 36 and 37, and, as demonstrated above, Compounds 36 and 37 showed no significant antagonist activity for the human MCH1 receptor. Since the final products are not active, I believe that the intermediate, Compound 31 would be expected inactive.

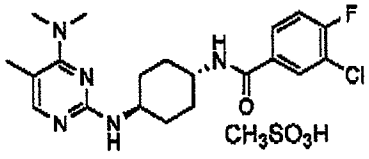
6. I believe the compounds 36 and 37 of "Wustrow" are not true homologs or isomers of the compounds in the present invention, because one of the structurally critical feature of the present invention is the configuration concerning the central cyclohexane ring of moiety L (formula (I)) which is 1,4-cis-cyclohexyl (formula (VII)).

In order to show the criticality of the configuration in the present invention, I conducted the following experiments to compare simultaneously the compound in the present invention "Example 3398" with the reference compound "trans-Example 3398" with respect to the antagonist activity for the human MCH1 receptor. The reference compound "trans-Example 3398" has the same chemical structure as "Example 3398"

except that the configuration concerning the central cyclohexane ring of moiety L is 1,4-trans-cyclohexyl (See the chemical structures below).



Example 3398



trans-Example 3398

7. Experimental Example 2

Method

HEK293 cells stably expressing constitutively activated the human MCH1 receptor were seeded $3x10^4$ cells/100 μL of complete culture media (Dulbecco's modified Eagle medium with 10 % fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.5 mg/mL G418) into poly-D-lysine pretreated black/clear bottom plates 24 hrs before the assay. Prior to the assay, the media were removed and HEK293 cells were loaded with 2 μM Fluo-4/AM calcium-sensitive dye in loading buffer (Hank's balanced salt solution supplemented with 0.5 mM probenecid, 0.05 mg/mL Amaranth, 20 mM HEPES, pH 7.4, and 0.2 % bovine serum albumin) for 1 hr at 37 °C in a 5 % CO_2 incubator. The loading buffer was removed, and fresh buffer containing various concentrations of the compounds of Example 3398 or the trans isomer, trans-Example 3398 was added to the cells, and the cells were incubated for 30 min at 37 °C. Fluorescence emission caused by increases in intracellular calcium mobilization elicited by 50 nM MCH was measured with FDSS6000™ system.

Result

The compound in the present invention (1,4-cis-cyclohexyl, "Example 3398") demonstrated remarkable antagonist activity for the human MCH1 receptor (IC_{50} = 1.7 nM). In contrast, the reference compound (1,4-trans-cyclohexyl, "trans-Example 3398") did not show any significant antagonist activity for the human MCH1 receptor at a concentration of 1 μM (IC_{50} > 1 μM).

Compound	IC_{50} (nM)
Example 3398	**1.7**
trans-Example 3398	**> 1000**

8. I further state that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: July 22, 2008

Kosuke Kanuma